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**Bulletin 85/44**(72) Inventor: **Kelder, Jan, Beethovengarde 97, NL-5344 CD Oss (NL)**  
Inventor: **Verheul, Hermanus Antonius Maria, Van Sonsbeeckstraat 10, NL-5344 JB Oss (NL)**(84) Designated Contracting States: **AT BE CH DE FR GB IT LI LU NL SE**(74) Representative: **van 't Holt, Hendrik et al, Postbus 20, NL-5340 BH Oss (NL)**(54) **Steroids for use as immunomodulators.**

(57) The invention relates to a group of  $\Delta^4$ - and  $\Delta^{5(10)}$ -oestrene derivatives having an alkyl(1-4 C) substituent in position 6 or 7, an oxy substituent in position 17 $\beta$  and optionally an oxo- or oxy-substituent in position 3 and a hydrocarbyl(1-4 C) group in position 17 $\alpha$ , for use as immunomodulator. The compounds are in particular suitable for therapeutic use in the treatment of autoimmune diseases.

**EP 0 159 739 A1**

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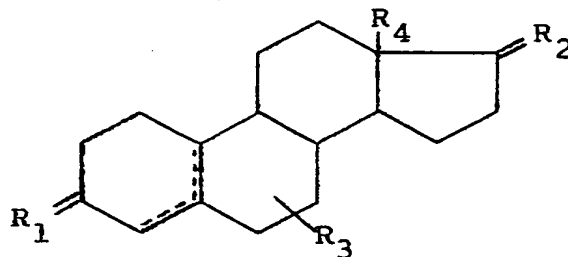
## STEROIDS FOR USE AS IMMUNOMODULATORS.

The invention relates to steroids of the oestrane series, in particular  $\Delta^4$ - and  $\Delta^{5(10)}$ -oestrene derivatives having an alkyl substituent in position 6 or 7, for use as immunomodulators, and to pharmaceutical preparations which contain these steroids as the active constituent. The invention also relates to prophylactic and therapeutic methods of treating auto-immune diseases in mammals.

Oestrene derivatives having a double bond between carbon atoms 4 and 5 or 5 and 10 and an alkyl substituent in position 6 or 7 are known. See, for example, British Patents 884 412, 885 782, 919 575, 931 009, 941 634, 965 292, 1 087 598, 1 104 462, 1 107 553 and 1 292 201, U.S. Patents 2 891 078, 3 012 045, 3 301 879, 3 341 557, 3 515 719 and 4 412 993, Netherlands Patent Applications 300 903 and 68.03 328 and Japanese Published Patent Application 21 373/63 (Dw 9532F).

Of the oestrene derivatives described in the literature, to which the present patent application relates, it is reported that they possess hormonal properties, in particular androgenic, anabolic, oestrogenic, progestative and/or ovulation-inhibiting properties.

Surprisingly, it has now been found that a certain group of  $\Delta^4$ - and  $\Delta^{5(10)}$ -oestrene derivatives having an alkyl substituent in position 6 or 7 possesses immunomodulating properties. The present invention therefore relates to oestrene derivatives having the general formula I



I

wherein

R<sub>1</sub> = H<sub>2</sub>, H(OR<sub>5</sub>) or O;

R<sub>2</sub> = (αR<sub>6</sub>)(βOR<sub>7</sub>);

R<sub>3</sub> = alkyl(1-4 C) in position 6 or 7;

R<sub>4</sub> = alkyl(1-4 C);

R<sub>5</sub> = H or acyl(1-18 C);

R<sub>6</sub> = H or hydrocarbyl(1-4 C);

R<sub>7</sub> = H or acyl(1-18 C); and

the broken lines indicate the presence of a double bond in the 4,5- or 5,10-position,

for use as immunomodulators.

The invention also relates to pharmaceutical preparations having immunomodulating properties, which preparations contain at least one of the compounds having the formula I.

By alkyl(1-4 C) there is meant methyl, ethyl, propyl, isopropyl, butyl, isobutyl or t-butyl.  $R_3$  is preferably methyl and moreover is preferably located in the  $\alpha$ -position.  $R_4$  is preferably methyl.

5        Acyl(1-18 C) is, as the affix (1-18 C) already indicates, derived from an organic carboxylic acid having 1-18 carbon atoms. As examples thereof there may be mentioned: formic acid, acetic acid, propionic acid, butyric acid, isobutyric acid,  
10       trimethylacetic acid, valeric acid, caproic acid, capric acid, pelargonic acid, undecylenic acid, lauric acid, palmitic acid, oleic acid, phenyl-acetic acid, phenylpropionic acid, cyclopentyl-propionic acid, cyclohexylcarboxylic acid, cyclo-  
15       octylacetic acid, benzoic acid, fumaric acid, maleic acid, succinic acid and citric acid.

By hydrocarbyl(1-4 C) there is meant one of the groups referred to above as alkyl(1-4 C) or an unsaturated version thereof, having 2-4 carbon  
20       atoms, such as vinyl, ethynyl, allyl, propargyl, isopropenyl, butynyl or butadienyl.

$R_6$  is preferably ethynyl, with  $R_7$  then preferably being H.

The compounds having the formula I and the  
25       pharmaceutical preparations based thereon have, as stated, immunomodulating properties, that is to say they are capable of exerting a favourable influence on the immune system in mammals. They are in particular suitable for therapeutic use in  
30       the treatment of auto-immune diseases, such as SLE (systemic lupus erythematosus), rheumatoid arthritis, auto-immune thyroiditis, Sjögren's syndrome, idiopathic thrombocytopenic purpura and haemolytic anaemia. The compounds can also be used  
35       prophylactically.

They can be delivered orally, rectally, parenterally or locally (topically or sublingually), usually in combination with a carrier and other pharmaceutical auxiliaries.

5           The compounds to be used in the present invention are known from the abovementioned patent specifications or can be prepared in a manner similar to that described in the said patent specifications.

10           Examples of steroids having the formula I, which may be used according to the invention, are:  
7 $\alpha$ -methyl-17 $\alpha$ -ethinyl-17 $\beta$ -hydroxy- $\Delta^{5(10)}$ -oestren-3-one, 7 $\alpha$ -methyl-17 $\alpha$ -ethinyl- $\Delta^{5(10)}$ -oestren-17 $\beta$ -ol, 7 $\alpha$ -methyl-17 $\alpha$ -ethinyl-17 $\beta$ -hydroxy- $\Delta^4$ -oestren-3-one,  
15 7 $\alpha$ -methyl-17 $\alpha$ -ethyl-17 $\beta$ -hydroxy- $\Delta^{5(10)}$ -oestren-3-one, 7 $\alpha$ -methyl-17 $\alpha$ -ethinyl- $\Delta^{5(10)}$ -oestrene-3 $\alpha$ ,17 $\beta$ -diol, 7 $\alpha$ -methyl-17 $\alpha$ -ethinyl- $\Delta^{5(10)}$ -oestrene-3 $\beta$ ,17 $\beta$ -diol, 7 $\alpha$ -methyl-17 $\alpha$ -allyl-17 $\beta$ -hydroxy- $\Delta^{5(10)}$ -oestren-3-one, 7 $\alpha$ ,17 $\alpha$ -dimethyl-17 $\beta$ -hydroxy- $\Delta^4$ -oestren-3-one,  
20 7 $\alpha$ -methyl-17 $\alpha$ -ethinyl- $\Delta^4$ -oestrene-3 $\beta$ ,17 $\beta$ -diol, 6 $\alpha$ -methyl-17 $\beta$ -hydroxy- $\Delta^4$ -oestren-3-one, 6 $\beta$ -methyl-17 $\beta$ -hydroxy- $\Delta^4$ -oestren-3-one, 6 $\alpha$ -methyl-17 $\alpha$ -ethinyl-17 $\beta$ -hydroxy- $\Delta^4$ -oestren-3-one, 6 $\alpha$ -methyl-17 $\alpha$ -ethyl-17 $\beta$ -hydroxy- $\Delta^4$ -oestren-3-one, 6 $\alpha$ -methyl-17 $\alpha$ -ethinyl- $\Delta^4$ -oestren-17 $\beta$ -ol, 6 $\alpha$ -methyl-17 $\alpha$ -ethinyl-17 $\beta$ -hydroxy- $\Delta^{5(10)}$ -oestren-3-one, 6 $\alpha$ -methyl-17 $\alpha$ -allyl-17 $\beta$ -hydroxy- $\Delta^{5(10)}$ -oestren-3-one and esters thereof.  
25 Particularly effective are the compounds 7 $\alpha$ -methyl-17 $\alpha$ -ethinyl-17 $\beta$ -hydroxy- $\Delta^{5(10)}$ -oestren-3-one  
30 (tibolone) and the corresponding 17 $\alpha$ -ethyl compound.

          The pharmaceutical preparations for use according to the invention can be prepared in accordance with known galenical techniques, for example by converting the relevant steroid compound  
35 having the formula I into a form which is suitable

for enteral (for example oral or rectal), parenteral (e.g. intravenous, subcutaneous or intramuscular) or local (via the skin or mucous membranes) administration. For this purpose, the steroid compound is mixed with or dissolved in a pharmaceutically acceptable carrier.

Examples of such preparations are tablets, pills, treats, coated tablets, pastilles, suppositories, powders, (micro-)capsules, emulsions, suspensions, solutions, implants, ointments, creams and lotions.

The quantity of active compound ( $\Delta^4$ - or  $\Delta^{5(10)}$ -oestrene derivative according to formula I) in the preparations to be administered enterally is 0.01-25.0 mg and usually 0.1-5.0 mg. The daily dose is usually 1-3 dosage units. In the liquid and semi-soft dosage forms which are injected or are applied to the skin or mucous membranes, the concentration of active compound is approximately 0.005 to 20% by weight, usually 0.01 to 5% by weight. Injections are usually carried out with dosage units of 1 ml.

The pharmaceutically acceptable carriers can be composed of one or more of the following ingredients: starch (for example potato starch or corn starch), sugars (for example lactose), lubricants (magnesium stearate or stearic acid), binders (for example amylopectin or polyvinylpyrrolidone), water, alcohol, glycerol and derivatives thereof, vegetable, animal and mineral oils and fats, fatty alcohols, silicones, lanolins, polyalkylene glycols, cellulose derivatives, silica, dispersants, emulsifiers, surfactants, anti-oxidants, preservatives, etc.

The immuno-modulating properties of compounds according to formula I are revealed by means of a test which is representative of the said action, namely the Bursa model.

5 In this model, the effect of the compound to be investigated on the development of the Fabricius bursa is investigated in chicks. The compound is administered in the embryonic stage by dipping  
10 eggs which have been incubated for three days in an ethanol solution of the compound to be investigated. The eggs are thereafter incubated further and ten days after the eggs have hatched, the chicks are killed and the body weight and weight of the Fabricius bursa are determined. The Fabricius bursa  
15 is an organ which is located immediately next to the cloaca and is responsible for the maturing of the B-cell system, which ensures the production of antibodies. The inhibiting action of the compound to be investigated on the development of the said  
20 organ is determined, using nandrolone (19-nor-testosterone) as reference.

The use of nandrolone as reference is based on the fact that nandrolone, in another model, the so-called NZB/W model, has an advantageous effect  
25 on lupus in NZB/W mice (murine lupus), which is an indication of an advantageous effect of this compound on auto-immune diseases. See, in this context, Clin.Exp.Immunol. 44 (1981), pages 11-17, in which  
30 article a comparison is made between the effect of testosterone and nandrolone in the NZB/W model, the conclusion being that the advantageous effect on "murine lupus" does not show any correlation with the hormonal action, namely with the androgenic  
35 action in the case in question.

A difficulty of the NZB/W model is that the tests in this model require such a long time (1 to 1½ years). For screening a large number of compounds, the Bursa model is to be preferred.

5 The results of the tests in the Bursa model are summarised in the following table.

	Compound	P
10	7 $\alpha$ -methyl-17 $\alpha$ -ethinyl-17 $\beta$ -hydroxy- $\Delta^{5(10)}$ -oestren-3-one	250
	7 $\alpha$ -methyl-17 $\alpha$ -ethyl-17 $\beta$ -hydroxy- $\Delta^{5(10)}$ -oestren-3-one	660
	7 $\alpha$ -methyl-17 $\alpha$ -ethinyl- $\Delta^{5(10)}$ -oestrene- 3 $\alpha$ ,17 $\beta$ -diol	20
15	7 $\alpha$ -methyl-17 $\alpha$ -ethinyl- $\Delta^{5(10)}$ -oestrene- 3 $\beta$ ,17 $\beta$ -diol	26
	7 $\alpha$ -methyl-17 $\alpha$ -ethinyl-17 $\beta$ -hydroxy- $\Delta^4$ -oestren-3-one	120
20	6 $\alpha$ -methyl-17 $\alpha$ -ethinyl-17 $\beta$ -hydroxy- $\Delta^{5(10)}$ -oestren-3-one	60
	6 $\alpha$ -methyl-17 $\alpha$ -ethinyl-17 $\beta$ -hydroxy- $\Delta^4$ -oestren-3-one	25
	6 $\alpha$ -methyl-17 $\alpha$ -ethyl-17 $\beta$ -hydroxy- $\Delta^4$ -oestren-3-one	150
25	6 $\alpha$ -methyl-17 $\alpha$ -ethinyl- $\Delta^4$ -oestren-17 $\beta$ -ol	89
	6 $\alpha$ -methyl-17 $\beta$ -hydroxy- $\Delta^4$ -oestren-3-one	15
	nandrolone (reference)	1

30 Column P indicates the potency of the compound in comparison with that of nandrolone. The values found appear not to correlate with the endocrinological activities of these compounds.



The effect of the compound 7 $\alpha$ -methyl-17 $\alpha$ -ethynyl-17 $\beta$ -hydroxy- $\Delta^{5(10)}$ -oestren-3-one (tibolone) was studied extensively in the NZB/W mouse model. NZB/W F-1 hybrid mice spontaneously develop an auto-immune disease with symptoms resembling those of human SLE and also develop Sjögren's syndrome-like disorders in the salivary and lacrimal glands. Tibolone inhibited significantly the expression of SLE and Sjögren's syndrome-like disorders in the NZB/W mice. For instance, mortality was reduced significantly. The median survival of female mice increased from 40 weeks (placebo) to 68 weeks with a dose of 0.1 mg/mouse/day and of castrated male mice from 54 weeks (placebo) to 74 weeks. The same dose significantly delayed the development of proteinuria. Also, the mononuclear cell infiltrations in the submandibular glands, expressed as the so-called infiltration index, were reduced from about 55 to below 10 in 39 weeks old NZB/W mice. Further, tibolone caused significant reduction of the auto-antibody (anti-dsDNA) levels in the serum of these mice.

The invention is further illustrated by the examples which follow. In each of the examples, the active compound can, if desired, be replaced by an equivalent quantity of another compound within the scope of formula I.

Example 1Tablets

	a)	7 $\alpha$ -methyl-17 $\alpha$ -ethinyl-17 $\beta$ -hydroxy- $\Delta^{5(10)}$ -oestren-3-one	2.5 mg
5		potato starch	10.0 mg
		magnesium stearate	0.5 mg
		ascorbyl palmitate	0.2 mg
		amylopectin	2.0 mg
		lactose to make up to	100.0 mg
10	b)	7 $\alpha$ -methyl-17 $\alpha$ -ethyl-17 $\beta$ -hydroxy- $\Delta^{5(10)}$ -oestren-3-one	2.0 mg
		corn starch	10.0 mg
		stearic acid	1.0 mg
		silica ("Aerosil")	1.0 mg
15		polyvinylpyrrolidone	3.0 mg
		dl- $\alpha$ -tocopherol	0.1 mg
		lactose to make up to	100.0 mg
	c)	7 $\alpha$ -methyl-17 $\alpha$ -ethinyl-17 $\beta$ -hydroxy- $\Delta^4$ -oestren-3-one	5.0 mg
20		potato starch	25.0 mg
		magnesium stearate	4.0 mg
		dl- $\alpha$ -tocopherol	0.2 mg
		lactose to make up to	100.0 mg

Example IIInjection preparations

	a)	<u>Suspension</u>	
		6 $\alpha$ -methyl-17 $\alpha$ -ethyl-17 $\beta$ -hydroxy- $\Delta^4$ -oestren-3-one	1.0 mg
30		Na carboxymethylcellulose	5.0 mg
		sodium bisulphite	0.2 mg
		water to make up to	1.0 ml

35

## b) Oil solution

6 $\alpha$ -methyl-17 $\alpha$ -ethinyl- $\Delta^4$ -oestren-17 $\beta$ -ol

2.0 mg

BHA/BTA (1/1)

0.2 mg

5

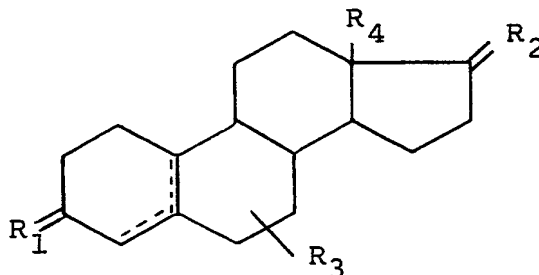
groundnut oil to make up to

1.0 ml

1.

CLAIMS

1. Steroids of the oestrane series, having the general formula:



wherein

$R_1 = H_2, H(OR_5) \text{ or } O;$

$R_2 = (\alpha R_6)(\beta OR_7);$

$R_3 = \text{alkyl}(1-4 \text{ C}) \text{ in position } 6 \text{ or } 7;$

$R_4 = \text{alkyl}(1-4 \text{ C});$

$R_5 = H \text{ or acyl}(1-18 \text{ C});$

$R_6 = H \text{ or hydrocarbyl}(1-4 \text{ C});$

$R_7 = H \text{ or acyl}(1-18 \text{ C}); \text{ and}$

the broken lines indicate the presence of a double bond in the 4,5- or 5,10-position,

for use as immunomodulators, particularly for use to combat or prevent auto-immune diseases.

2. Steroids according to claim 1, wherein  $R_1 = 0$ .
3. Steroids according to claim 1 or 2, wherein  $R_3 = \text{methyl}$ .
4. Steroids according to claims 1-3, wherein  $R_3 = \alpha\text{-methyl}$ .
5. Steroids according to claims 1-4, wherein  $R_4 = \text{methyl}$ .
6. Steroids according to claims 1-5, wherein  $R_6 = \text{ethynyl}$  and  $R_7 = \text{H}$ .
7. A steroid according to claim 1, which is  $7\alpha\text{-methyl-}17\alpha\text{-ethynyl-}17\beta\text{-hydroxy-}\Delta^{5(10)}\text{-oestren-3-one}$ .
8. A steroid according to claim 1, which is  $7\alpha\text{-methyl-}17\alpha\text{-ethyl-}17\beta\text{-hydroxy-}\Delta^{5(10)}\text{-oestren-3-one}$ .
9. A pharmaceutical preparation having immunomodulating properties, which contains a compound according to claims 1-8.



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# EUROPEAN SEARCH REPORT

0159739  
Application number

EP 85 20 0423

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
X	THYMUS, vol. 3, no. 2, 1981, pages 105-117, Elsevier/North-Holland Biomedical Press, Amsterdam, NL; A.B. McCRUDEN et al.: "Androgen binding cytosol receptors in the rat thymus: physicochemical properties, specificity and localisation" * Whole article *	1-9	A 61 K 31/565
D,A	GB-A- 885 782 (G.D.SEARLE) * Claims 1-9; page 1, lines 29-35 *	1	
D,A	GB-A- 884 412 (ORGANON) * Claims 4-6; page 1, lines 19,20 *	1	
D,A	GB-A- 931 009 (ORGANON) * Claims 5-9; page 2, lines 107-110 *	1	TECHNICAL FIELDS SEARCHED (Int. Cl.4) A 61 K 31/00
D,A	GB-A-1 107 553 (ORGANON) * Claims 3-8; page 2, lines 80-84 *	1	
D,A	GB-A- 941 634 (THE UPJOHN CO.) * Claims 1-20; page 7, lines 4-18 *	1	
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 25-06-1985	Examiner HENRY J.C.
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

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# EUROPEAN SEARCH REPORT

0159739

Application number

EP 85 20 0423

Page 2

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
D,A	US-A-3 301 879 (ALBERT WETTSTEIN et al.) * Claims; column 1, lines 57-71 *	1	
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D,A	GB-A-1 292 201 (ORGANON) * Claims 4-8; page 1, lines 54-57 *		
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D,A	US-A-3 012 045 (F.B. COLTON) * Claims; column 1, lines 31-47 *	1	
---			TECHNICAL FIELDS SEARCHED (Int. Cl.4)
A	GB-A-1 104 462 (ORGANON) * Claims 4-7; page 2, lines 97-108 *	1,7,8	
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A	FR-A-1 583 441 (UPJOHN) * Abstract; page 1 *	1	
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A	US-A-4 412 993 (J.H. SOKOLOWSKI) * Whole document *	1	
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The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 25-06-1985	Examiner HENRY J.C.
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone		T : theory or principle underlying the invention	
Y : particularly relevant if combined with another document of the same category		E : earlier patent document, but published on, or after the filing date	
A : technological background		D : document cited in the application	
O : non-written disclosure		L : document cited for other reasons	
P : intermediate document		& : member of the same patent family, corresponding document	

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